FACTORS INFLUENCING ODOR SENSITIVITY IN THE DOG

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CONTENTS

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SUMMARY

This report covers the following studies: comparison of odor detection performance in man and dog; development of a technique for the quantitative analysis of sniffing patterns in the dog, the enhancement of a dog's performance on an odor detection task by oral administration of the same odor prior to testing and the correlation between circulating progesterone levels and performance in an odor detection task in female rats.

Detection curves were previously derived for alpha-ionone for four German Shepherd dogs. To determine the generality of those findings, six men and one woman were tested in the same apparatus using comparable procedures and the same compound. Human detection curves reach their asymptotes in one and half log units of concentration while those of dogs extend over five log units. The marked discontinuity in the slope of the canine detection curves was also seen in the curves for four of the human subjects and in one case extends over 50% of the dynamic range. Three subjects showed no discontinuity.

Human thresholds for alpha-ionone fell at a mean concentration of 4.5×10^{9} molecules/cm^3 while canine thresholds were at 4.5×10^{5} molecules/cm^3.

A technique has been developed for the quantitative analysis of the relation between odor detection task and sniff parameters. Thirsty dogs are rewarded with water for identifying which of two ports is associated with an odor. Sniff flow rate, frequency and amplitude are recorded from the output of a pneumotachometer behind one port. The nature of the change in sniff parameters that accompanies change in odor concentration is being investigated. This will also allow an estimate of the number of molecules entering the nasal chamber at each sniff.

Alterations in the detection of alpha-ionone have been investigated following oral administration of 1 ml alpha-ionone. Dogs were trained to criterion on $10^{-4.5}$ alpha ionone and a baseline performance established before the compound was administered. Following administration performance rose to oscillate above baseline. It reached a peak at 12 percentage points above baseline on the 7th day before declining to baseline on the 10th day. The peak far exceeded the performance score that the dog previously achieved at this concentration indicating that a significant enhancement of performance can be induced by this method.

The performance of female rats on an odor detection task was previously shown to vary according to the phases of the estrus cycle. In an attempt to determine the relation of circulating plasma levels of gonadal steroids to this cyclicity the correlation between each of the hormones progesterone A, progesterone B, and progestin, and performance was determined. The highest correlation (.999) occurred with progesterone A. Decreasing performance levels are associated with increasing levels of the hormone.

PART I

ODOR DETECTION PERFORMANCE IN THE DOG

A. ODOR DETECTION PERFORMANCE IN DOG AND MAN

Introduction

We have developed a technique for investigating odor detection in the dog and used it to derive detection curves for α-ionone using four German Shepherds (see previous reports). These results gave information concerning the form of the curves; the absolute detection threshold of the dog and certain of the factors which control odor detection performance. The form of the curves is remarkable in that it shows marked reversals or discontinuities in slope in the top third segment. Since this may reflect the action of two different types of receptor it is important to know if the reversals are peculiar to the dog or seen in other species. The low detection threshold for alpha-ionone also indicates the need for information on other species, particularly man. If the dog is unusual in this respect its value in detection tasks would be confirmed. If however, the performance is not remarkable as compared with man then it raises the possibility of developing more efficient methods for training human subjects to reduce reliance on dogs. Finally, it would be valuable to know whether some of the variables controlling the dogs' performance also apply to human subjects.

For these reasons we have derived detection curves for alpha-ionone using human subjects and the same apparatus and testing procedures as were used in the previous study with dogs. We believe the procedures and techniques that we have applied overcame certain deficiencies present in earlier attempts to compare human and canine detection performance. In particular, these studies did not involve direct comparisons derived from the same apparatus and lacked two or more of the following: (1) accurate olfactometric techniques (including an independent source of calibration such as a gas chromatograph); (2) adequately trained and motivated dogs; (3) adequately trained and motivated human subjects; (4) controls for cues that could be used by the animal to make a correct choice (for example, transfer of cues from handler to dog).

Methods

(a) Odorant

The alpha-ionone used was from two sources: (1) for all first series trials with dogs involving concentrations of 10^{-6} and higher, the supplier was K & K Laboratories Inc. (Plainville, New York). The stated purity was 95-99%. (2) for all remaining trials including those involving human subjects, the supplier was Givaudan Corporation (Clifton, New Jersey). The lot number was #3407-72 (Irison, alpha) and the stated purity was 99.2%. The gas chromatographic analysis supplied by the manufacturers is reproduced in Figure 1. Alpha-ionone is the main peak visible but there are also minor peaks.

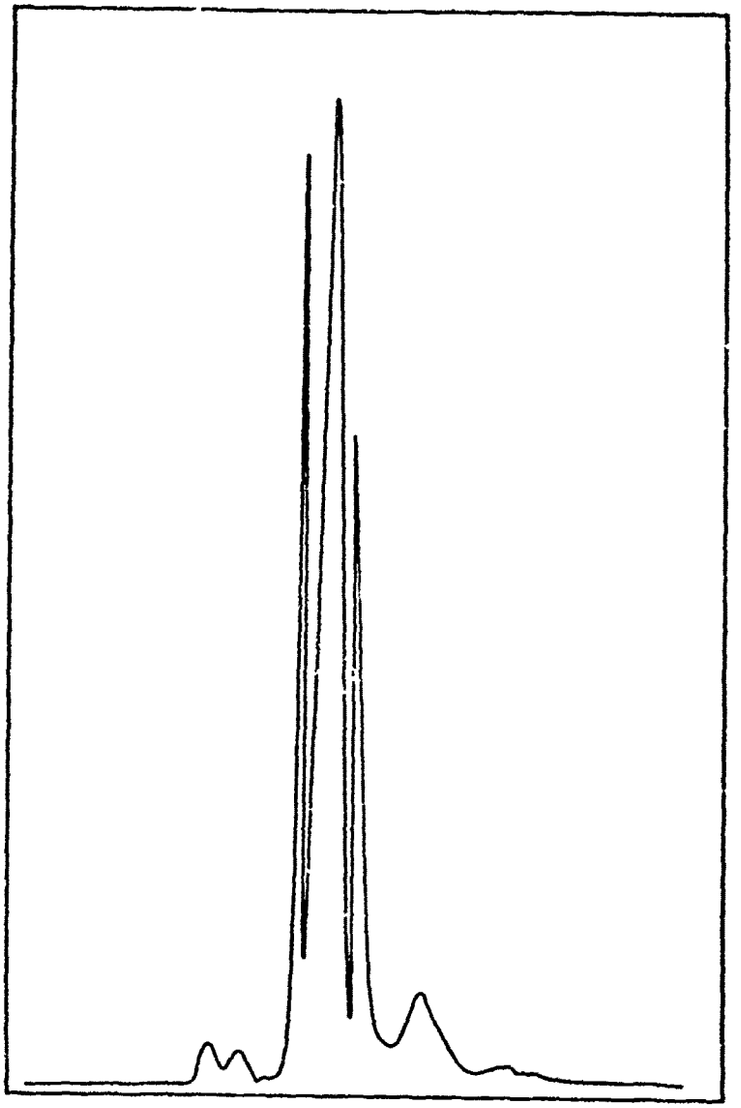

Fig. 1. Gas chromatograph of α-ionone sample. The main peak is attenuated. (The sharp side peaks are the non-attenuated segments of the main peak.) The peaks on the baseline are impurities and consist of three major and 2-3 minor deflections. Purity = 99.2%.

(b) Olfactometer and its calibration

The olfactometer dilutes by combining the odorized air with a known volume of purified air at each of up to six dilution stages. Because of the small volumes of odor involved, the accuracy of the delivered concentration depends heavily on the accuracy of the first dilution step and, in particular, on the degree of saturation achieved and the accuracy of the flowmeters. Since this is critical in obtaining reliable estimates of absolute threshold and establishing the validity of discontinuities in the slope of the odor detection curves, we have investigated the characteristics of the first output stage of the olfactometer with a Varian 1520 gas chromatograph. In the process we have also determined the optimal flow rate to achieve saturation, the time required for equilibrium to be established at the final output of the olfactometer and the stability of this output.

Chance level

Since dogs were faced with three bays, the apparent theoretical chance level over a series of trials is 33.3% correct. The number of times that an odor appeared in the same position in successive trials was programmed never to exceed three. When three successive presentations in the same bay were reached, however, the theoretical chance level in the next trial was, in fact, 50% (i.e., a 50% chance that the odor would appear in one of the two remaining bays). There is the possibility that the dog's response biases could have established a level slightly different from 33.3%.

We used two methods to gain additional information on this point. The first involved analyzing the actual chance score from the sequence on the paper programmer over blocks of 50 trials. The second was to determine the performance of dogs on "blank" runs which were generated when the effectiveness of olfactometer cleaning procedures were assessed following a change in concentration. The results of these methods all fell within the range of 32-33%.

Training and testing procedures

Both apparatus and methods used for training and testing dogs have been described in previous reports. In brief, thirsty dogs are trained in a programmed odor-choice apparatus to sample each of three odor-air presentation bays and indicate (by the sustained interruption of a photocell beam) which of three bays is associated with an odor. If a correct choice is made the dog is rewarded with water delivered to a cup inside the bay. If incorrect, access to the bay is blocked. Each bay receives odor or filtered air from an air-dilution olfactometer.

In essence, the same procedures were used with human subjects. Water, however, was not a practical means of introducing a reward for human subjects. Instead, a coin dispenser was introduced that delivered

10¢ onto a ledge at the site of the main compartment of the box when a correct choice was made. A further problem arose with restrictions imposed by the size of the box. Subjects were therefore allowed to kneel or sit in any way that was comfortable. Finally, the odor or air was delivered to a bay which was difficult for human subjects to reach. To overcome this, subjects held a 10" teflon-covered cone that was shaped to fit over the nose at one end and could be applied to the perforated outlet disc on the floor of the bay, at the other end. They were given a sheet of instructions to read before beginning the series of trials. This included a suggestion that they try different sniffing procedures until they achieved one which seemed to yield maximum performance. There was no restriction on the duration of sampling at any one bay. When a subject made a choice, he interrupted the beam in the chosen bay for 3 seconds.

Initial trials showed that subjects differed in the duration of their sampling time at each bay. Since this could influence performance three subjects were asked to time the duration of their sampling period with a stopwatch. (The sampling period was defined as the time elapsing between the opening of the bay doors and the making of a choice. It did not include the inter-trail interval.) The average time taken was averaged over each session of 15 trials and compared with actual performance.

Concentrations were presented in a descending series as in the dog study. Instead of 1/2 or 1 log unit concentration steps, however, 1/4 log steps were presented since the dynamic range of the detection curve proved to be smaller. Subjects were exposed to the highest concentration tested (10^{-3} of saturation) to familiarize them with odor initially. Thereafter the first 15 trials of every concentration tested were discarded and only the subsequent 30-65 used.

Six men and one woman were tested and their performance compared with that of the four bitches measured in the previous study.

Results and discussion

Detection curves.

Figure 2 shows detection curves for alpha-ionone for three of the seven human subjects while Figure 3 compares the mean performance of all human subjects with that of the dogs. The higher variance of the human data may reflect the much smaller number of trials that were run rather than any real difference in variance of human and dog performances.

The human detection curves reach their asymptotes in about 1 1/2 log units of concentration while canine detection curves cover about 5 log units. On the other hand, most of the dynamic range of both curves falls within 1 1/2 log units of concentration and has a comparable slope. The curves also differ in the slope of the final segment of the curve as it approaches threshold. In the case of the canine curves the approach

is more gradual than the rapid drop-off seen in human curves.

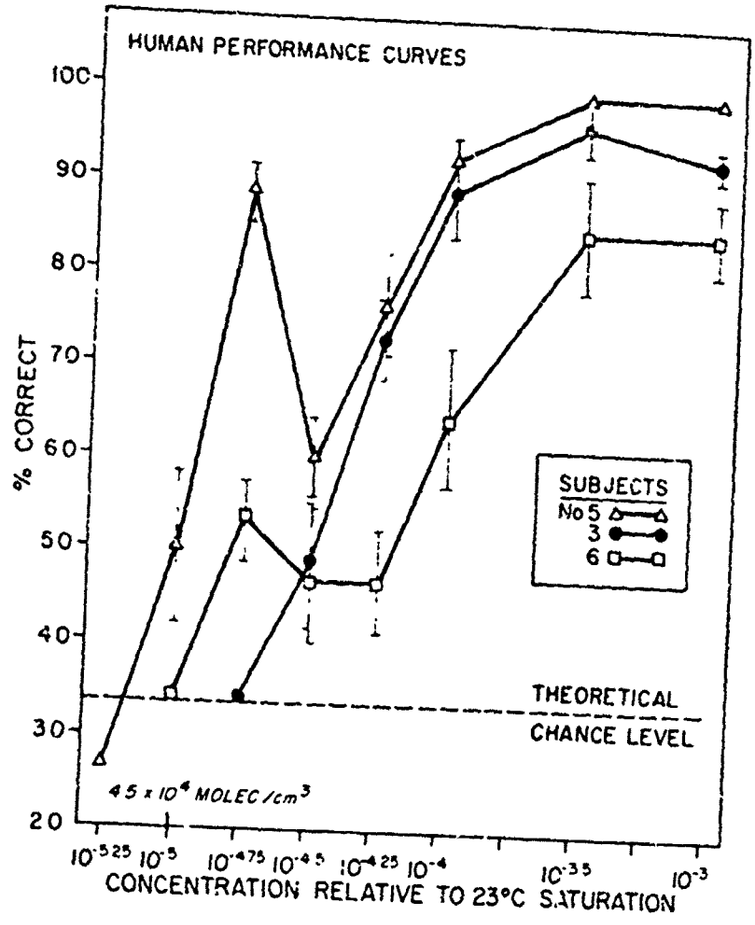

Fig. 2. Detection curves for α-ionone in three male human subjects ($4.5. \times 10^9$ moles/cm^3 refers to the mean detection threshold). Each point on the curve is the mean ($\pm$ SE) of at least 150 replications.

The main discontinuity in the slope of the canine detection curve was also seen in curves for three human subjects. It was particularly marked in one subject who showed a reversal extending over 50% of the dynamic range. The average point on the curve at which the reversal occurs is separated by about 20% on the performance scores. In contrast to the canine curves, three of the human subjects showed no significant discontinuity.

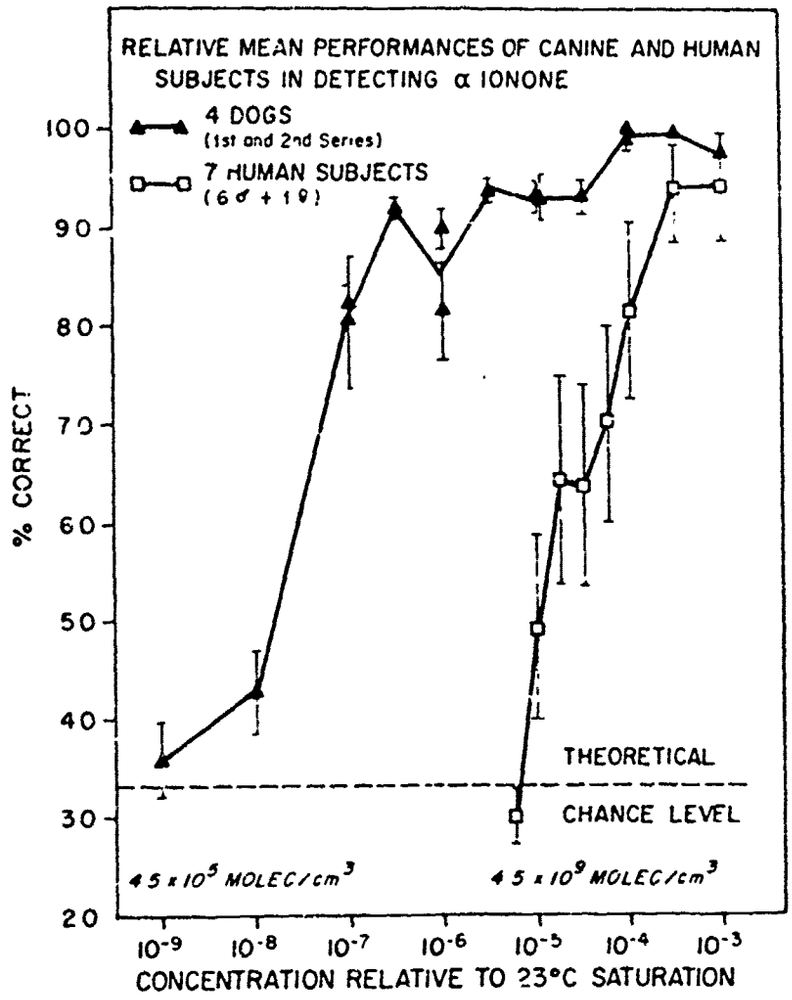

Fig. 3. Comparison of detection curves for alpha-ionone in dog and man. (Concentrations expressed in molecules/cm^3 refer to the absolute detection thresholds.)

A further point of dissimilarity is the absence in any human curves of significant reversals at other points in the curve. This probably reflects the absence of a gradually sloping upper limb to the curve which, in turn, may reflect the absence of performance scores above 95%. Such scores might have occurred had concentrations above 10^{-3} been tested on humans.

Absolute thresholds.

Human performances reach chance level at a mean concentration of 4.5×10^{9} molecules/cm^3. The canine thresholds reach threshold at about 4 log units lower than this - namely, 4.5×10^{5} molecules/cm^3. These differences, however, must be viewed with caution since canine performance is based on life-long training in detecting and responding to odor cues. Man, on the other hand, makes minimal use of his nose. Even within this study it was not possible to subject human subjects to the same sustained periods of training and the curves were derived from far fewer replications. These differences, therefore, are probably an exaggeration of the real difference - possibly by a log unit.

Relation of sampling time to performance

Human subjects varied from a few seconds to several minutes in the time they spent sampling bays before reaching a choice. At higher concentrations, sampling was generally rapid. Near threshold, however, subjects would occasionally take up to 4-5 minutes.

To determine whether longer sampling time was associated with a higher level of performance, correlation coefficients were calculated between mean sampling time and mean performance scores of all subjects for each concentration. The results show no significant correlations exist. Thus there is no support for the hypothesis that longer sampling times improve performance. No attempt was made to determine whether subjects achieved their sampling times by sniffing for prolonged periods at each bay without interruption or by sampling frequently among the bays.

Olfactometric calibration

Calibration of the olfactometer by gas chromatography indicated that odor saturation was not complete, possibly due to a slight cooling effect in the saturation. The dilutions estimated from flowmeter were in error by about 5 per cent.

Discontinuities in slope

The marked reversal in slope seen in the dog study also appears in the average curve for human subjects (Fig. 3). It is particularly marked in the curves for two of the three subjects in Fig. 2 but, unlike the dogs, one individual shows no reversal or discontinuity. The point at which it appears is lower in the curve than is the case with dogs.

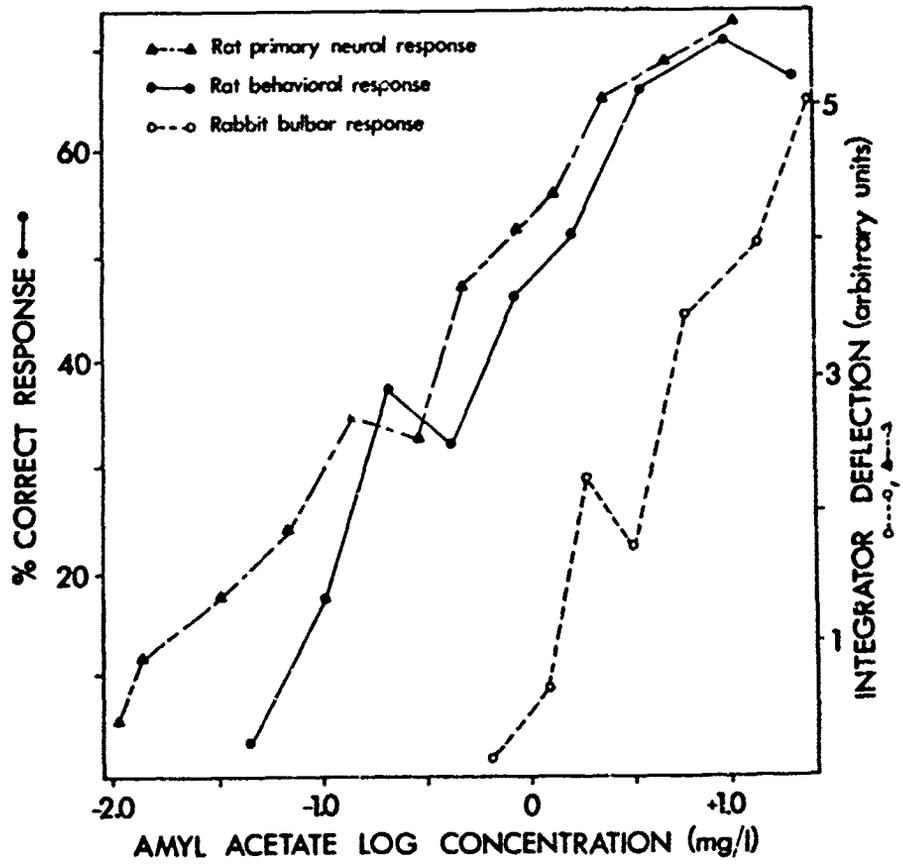

Fig. 4. Stimulus-response curves for amyl acetate obtained in three different studies to illustrate reversals of slope. Comparison between detection curve (obtained in rat by Moulton, 1960) with curve obtained in electrophysiological studies on bundles of rat primary olfactory neurones (Mathews, 1972) and rabbit olfactory bulb multi-unit responses (Mozell, 1958).

The phenomenon is thus not concentration-linked and so could not be due to instrumental artifacts. Such reversals have been noted previously (Fig. 4) in curves for amyl acetate. They occur in this case at the receptor level. This strengthens the view that in the present study the reversals reflect non-linear events generated either at the level of the receptor sites or - less probably - at the level of the impulse-generating mechanism. The simplest explanation is that two receptor site types exist.

One of these has a greater sensitivity to lower concentrations of alpha-ionone than does the other. At higher levels, however, its performance saturates and declines, unmasking the performance of the remaining site type. The combination is a bimodal distribution. If this is true it suggests that some individuals may not possess both site types.

B. A QUANTITATIVE ANALYSIS OF SNIFF RATE, AMPLITUDE AND FREQUENCY IN DOGS PERFORMING AN ODOR DETECTION TASK

Introduction

Assume we introduce a stimulating agent to an organism and the organism attends to it. In most sensory systems this agent has virtually continuous access to the receptor. For example, when we view this page the only mechanism that intercepts access of the pattern to the retina is the brief, insignificant blinking of the eyelids. In olfaction, however, odors can only reach the receptors intermittently: transport is largely under respiratory control.

The nature of this control is complex. We can see this in casual observation of a dog sniffing an odor source. Sometimes it sniffs in a burst of quick successive inhalations. Occasionally it sniffs with prolonged individual inhalations separated by vigorous exhalation down the nose. Successive sniffs may vary in amplitude and duration and the temporal patterning of sniffs within different bursts may differ. (One is reminded of the movements of a hand adjusting an object undergoing tactile exploration.)

Because of the complexity of this transport process in olfaction we may suspect that it has some important implications for understanding the way in which odors are detected and recognized. For esample, because of the tidal rise and fall of airflow odor concentration cannot normally reach equilibrium with the mucus covering the receptors. Does neural coding of olfactory information therefore incorporate mechanisms for detecting these changing concentrations? In fact, Kauer (1974) found units that are tuned to a limited concentration range in the salamander olfactory bulb. Furthermore, Macrides and Chorover (1972) concluded that entrainment of the response pattern of rat bulbar units to the respiratory cycle could be an important component coding for odor quality. But there may also be properties of the stimulus that can only be identified if equilibrium conditions are established. If so, what are these circumstances and does the dog make provision for achieving equilibrium when they exist?

The tidal movement of odors also has a spatial component. Much of the olfactory receptor sheet lies in blind-ending recesses. If there were different receptor types, each distributed randomly across this sheet it would not be important whether or not molecules had access to all parts of the sheet. But, in fact, certain regions are specially sensitive to odors tested - at least in the Tiger salamander (Kauer and Moulton, 1974). Presumably, there are mechanisms to ensure adequate stimulation of these

areas - mechanisms which may involve special manipulations of the respiratory airflow: in other words, sniffing. The tidal flow of odors also increases the difficulty of determining the accual number of molecules of an odorant reaching the receptors. This point is particularly relevant in assessing odor detection curves presented in the previous section. It also bears on the design of behavioral test apparatus as it relates to the rate of odor delivery. If an animal's sniff volume per unit time exceeds that delivered, dilution of the odor must be occurring.

These lines of evidence all point to the growing need for a quantitative analysis of the characteristics of airflow cycles while the subject is engaged in an odor detection task. No such analysis has been made except in relation to sniffing frequencies. This data was obtained by Welker (1964) who filmed vibrissae movements in the rat. He found the sniff to be a standardized movement occurring in bursts of 6-11 cycles/sec when the rat was exploring odors. When anticipating shock, however, the frequency may be as low as 1/2 cycle/sec (Clarke, 1970). These studies, however, provide no information about sniff volume or amplitude nor are they concerned about controlling the purity and nature of the odor source; the distance of the animal's snout from the source; or the effects of systematically varying concentration or odor quality on sniff parameters. The aim of the present study is therefore to provide such information for a dog performing a learned odor detection task.

Because of the need to measure flow rates with a high degree of accuracy a pneumotachometer was used. In preliminary trials this was incorporated in a face mask which the animal wore during trials. However, the weight of the device proved too much for the dog to work comfortably and we therefore devised a method in which the pneumotachometer was attached to a fixed frame. The dog was trained to respond differentially to the presence of an odor in such a way that it sniffs through the pneumotachometer. The ultimate aim is not only to measure accurately the flow amplitude, duration and frequency but also to determine whether these parameters vary as a function of concentration, nature of the odor and of the task (i.e., whether it is odor discrimination or detection). If the mode of dispersal of odors within the olfactory organ is important (for example, in discrimination rather than detection) it should be reflected in such data. Since the work is still in progress and data have not yet been analyzed this is a preliminary report.

Methods

Subjects.

Two female and one male German Shepherd were used. One female was about three years old at the start of the experiments while the other dogs were about one year old. They were housed in temperature controlled indoor runways, fed laboratory chow ad lib, and placed on a 23-hour water deprivation schedule. During testing and training they received an average of about 400-600 cc of water as rewards. The difference between this quantity and 1500 cc was given to them early each morning following the day

of testing.

Behavioral test apparatus

The apparatus provides two bays, one associated with the odor of amyl acetate, and the other a blank. The two bays are set in a wooden console. Two swinging metal doors carry the sniffing ports. They are counterweighted to allow the dog to push them open but can be latched in position to block the dog's access to the water bowls (visible beneath the doors). The experimenter releases the latch by remote control when the dog makes a correct choice. The bowls are gravity fed from calibrated water reservoirs in the upper section of the console.

Behind each sniffing port are two metal cylinders of similar length and diameter extended inwards by polyethylene cylinder. One of these is the Fleish pneumotachograph, the other is a dummy. To equalize flow resistance in the two cylinders the internal lumen of the dummy is fitted with a smaller cylinder. Since their relative positions can provide no differential cues, the cylinders occupy the same positions permanently. The cylinders are open at both ends but near the opening into the interior of the console there is a port on the floor of each polyethylene cylinder. This is made to accommodate a 7 cc cuvette, set so that its mouth is flush with the lumen of the cylinder. One cuvette contains pentyl acetate in the diluent while the other cuvette contains the diluent alone. The relative positions of the cuvettes are varied according to a randomly determined sequence.

Each of the sniffing ports is surrounded with a ring of foam rubber. This allows the dog to insert its snout into the port without irritation yet seals tightly enough to prevent air leaking around the dog's snout.

Odorant and concentration determination

N-pentyl acetate was chosen as the first odorant because it has previously been used in olfactory studies (on rats, rabbits, tortoises, pigeons and man) involving both electrophysiological and behavioral apparatus; has a sharp distinctive odor with a known trigeminal threshold lying well above the olfactory threshold and has no known biological significance for the dog. It was diluted with ethylene glycol (Baker reagent grade) to the appropriate concentration.

A preliminary estimate of the actual concentrations present in the head space above the odor was established by drawing off a sample and injecting it into a gas chromatograph (see Part IA). This indicated that a dilution of 10^{-3} in solution was equivalent to 10^{-5} of vapor saturation in the head space above the liquid.

To avoid any confusion stemming from these differences we will refer to concentration in solution as % concentration and concentration in air as a fraction of vapor concentration (10^{-3}, 10^{-4}, etc.).

Recording apparatus

The pneumotachograph is a device that measures flow rates with high accuracy and minimal resistance. The flow resistor elements are a large number of ducts (each 0.8 mm in diameter and 32 mm long) packed into a brass tube. When air is drawn through the outer layer of ducts the difference in pressure at two ports (separated from each other along the long axis of the tube) is used to measure flow. This pressure difference is translated into an electric signal by means of differential pressure transducer. (This has a strain gauge as one arm of a wheatstone bridge.) The signal from the pressure transducer is amplified and displayed on a pen recorder and oscilloscope or recorded on tape for later display.

To calibrate the pneumotachograph for volume a 1000 cc syring was fitted with an adaptor to attach it to the pneumotachograph, and air drawn through into the syringe. The amplitude of the pen deflection was then plotted against flow rate. To calibrate for flow rate flowmeters were used. Temperatures were relatively constant during trials and the volume of tidal air involved was small (in the order of 100 cc).

Training and testing

The procedures for training and testing were based on principles outlined in earlier reports. In essence, the dogs were required to respond differentially to the presence of the odor by pushing on the appropriate door with their snout. A novel feature, however, was the need to train dogs to insert their snouts into sniffing ports. Initially they were required to keep their snouts in the ports for 1-3 seconds. However, when this task was learned (and the dogs were responding differentially to the presence of the odor) they were allowed to set their own time since this was one of the variables under study.

Training began at concentrations of 10% until performance stabilized at 90% or more correct responses. The concentration was then lowered to 1% and the process repeated. The final concentration used for testing was .01% (corresponding to approximately 10^{-6} of vapor saturation).

Dogs sniffed into the port associated with the pneumatochograph both when the odor was present and when the diluent alone was present. Consequently the time required to make a correct choice could be identified.

Results

Dogs readily learned to sniff into the ports. While they were able to maintain this position for 1-3 seconds during training, all required considerably less time to make a choice when allowed to take as little or as much time as they required. This parameter showed much individual variability, however.

When a dog first inserted its snout into the port the pneumotachometer recordings showed an immediate downward deflection which probably

represents the displacement of air through the pneumotachometer by the snout rather than expiration of air.

Data for one dog show that sniffing bouts are usually structured around 1-3 trains with 3-7 sniffs per train. Occasionally one or two isolated sniffs may occur following the main train but the initial train invariably contains less than three sniffs.

Examples of data derived from responses to both air and odor are given in Table 1 and in Fig. 5. (In interpreting this data it should be borne in mind that the dog may sniff first from either the right port which is always associated with the pneumotachometer or the left port. Thus the initial train recorded may or may not be the initial train of a trial. Secondly, the dog may sample the left port between trains recorded here as a single bout. For the purposes of compiling this data a single sniff was counted whenever the trace fell below baseline.)

There is an insufficient number of responses to allow firm generalizations but there is some indication both here and in other records that the largest volume sniff occurs towards the end of a train - usually the last or penultimate sniff. Furthermore, sniffing trains generally begin rather tentatively with one or more low volume sniffs. Beyond these features and the tendency for frequencies to occur between 6.0-7.5/sec. there is little uniformity in the sniff pattern. Even the frequency figures are misleading in so far as they imply regular spacing of sniff cycles. Individual sniffs (inhalations) are separated by varying periods of exhalation or of minor fluctuations around the baseline and the durations of the sniffs themselves are highly variable.

Flow rates during individual sniffs range from 16-87 l/min. Peak flows, however, are only reached momentarily (<5 m sec duration). In one case, however, a flow rate of 50-56 l/min was sustained for 60 m sec. Volumes range from 5-220 cc/sniff and durations from 25-163 m sec. A sniff train varies from 0.438-1.138 secs. of which actual sniffing (inhalation) seldom accounts for much more than 1/2.

While it is too early to interpret this data in terms of the pattern of dispersion of odorants at olfactory surface, it is interesting to observe the frequency with which individual sniffs achieve their peaks - or decline from them - by a series of secondary peaks. This "jitter" should increase turbulence and thus may act in directing eddy currents into the more remote recesses of the olfactory chamber.

There is some indication that the dog spends longer (per unit volume sniffed) in investigating an odor associated than an air related port. For example, in Table 1 the ratio of Total Volume sniffed/total duration of trains is 0.35 for odor as against 0.45 for air. The time spent actually sniffing air and odor (per unit volume sniffed) is, however, similar. (The corresponding ratio is 0.85 for odor as against 0.83 for air.)

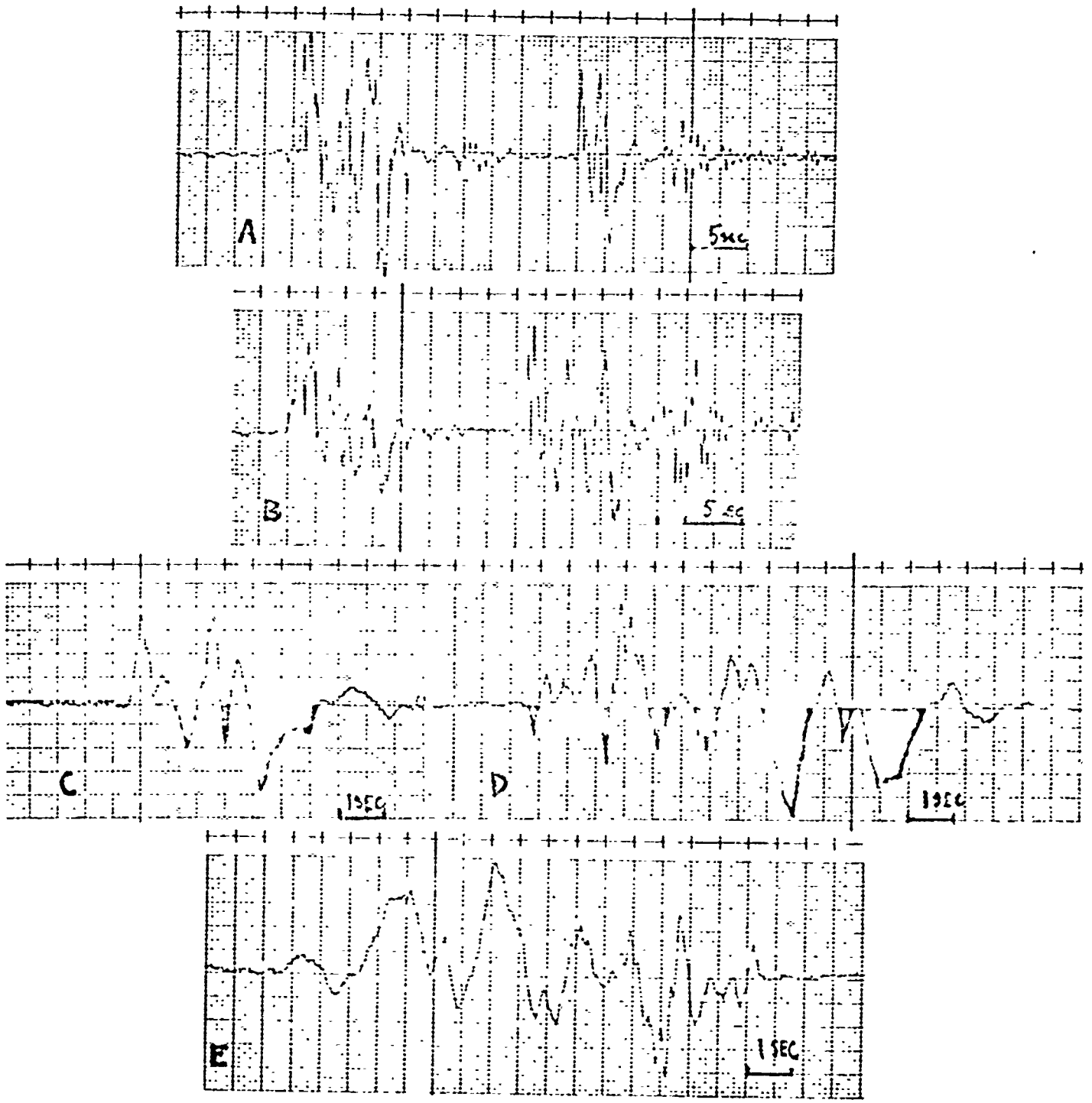

Fig. 5. Pneumotachogramps of sniffing trains (for explanation, see bottom of page 19).

When more data become available it whould be possible to determine whether sniff patterns, amplitudes or durations alter as a function of individual differences, of concentration or quality of the odor, or in sniffing odor as opposed to air, or whether flow rate, duration or volume are differentially emphasized at different phases of a sniff train. (For example, the present data already suggest that flow rate and duration are maximal relative to volume at early stages of a cycle while the reverse is true at later stages.)

Fig. 5. Pneumotachographs of sniffing trains.

In all records inhalations (sniffs) are shown as downward deflections and exhalations as upward deflections. (In traces C and D sniffs are shaded.) The odor in each case is air odorized by passing over 1% amyl acetate solution which corresponds to approximately 10^{-7} of vapor saturation at the point of sniffing (gas chromatograph calibration). A, B, and C: Odor. D and E: Air. Note that A and B are recorded at a less rapid speed than C, D, and E, and show two bursts each.

SNIFF TRAIN	TOTAL* DURATION (m. sec)	NUMBER OF SNIFFS IN TRAIN	FLOW RATE (l/min)	VOL. (cc)	DURATION (m. sec)	FREQUENCY (per sec)
1 AIR	975	1	22.0	10	38	6.1
		2	44.0	31	31	
		3	31.5	19	50	
		4	34.5	30	63	
		5	77.5	150	113	
		6	56.0	165	150	
2A ODOR	800	1	21.5	10	25	7.5
		2	44	63	100	
		3	16	5	25	
		4	44	75	100	
		5	87	220	163	
		6	31	40	63	
2B ODOR	500	1	34.5	30	56	6.0
		2	28	15	25	
		3	61	133	156	
3 AIR	438	1	16	10	35	6.8
		2	37.5	40	46	
		3	56	83	100	
4A ODOR		1	23	15	38	3.5
		2	31.5	58	103	
		3	59.5	160	153	
		4	59.5	53	150	
4B ODOR		1	79	140	125	
		2	56	95	125	
4C ODOR		1	68.5	185	150	

Table 1. Flow rate, volume and durations of sniffs drawn from over 0.1 per cent of amyl acetate in polypropylene glycol ("odor") and the diluent alone ("air").

*Total duration refers to elapsed time between start and finish of sniff train and includes exhalation as well as inhalation (sniff) time.

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C. ENHANCEMENT OF ALPHA-IONONE DETECTION FOLLOWING INGESTION OF ALPHA-IONONE

Introduction

In pilot studies (E. White and D. G. Mouton, unpublished) we found that when 1 ml alpha-ionone was injected systemically into a rat with electrodes chronically implanted in the olfactory bulb, the averaged multi-unit discharge in response to stimulation by alpha-ionone increased markedly over a four-day period. Since these experiments were done on anesthetized animals and did not allow any evaluation of the extent to which the animal's detection abilities were altered behaviorally, it seemed important to reexamine this phenomenon in dogs. There are several reasons for this choice. In particular, the fine tuning and stability of the dogs' performance on low concentration which we have now achiever, provides a unique and highly sensitive baseline. It would be expected that even slight alterations in response conditions could be immediately detected. For example, even the poorest performing dog showed a standard error of only $\pm$ 3.2 on an 89% performance at $10^{-4.5}$ alpha-ionone. Even on a session-to-session basis mean performances do not usually deviate significantly from this narrow range.

The present study was thus a preliminary attempt to determine whether the ingestion of an odorant enhances or depresses the ability to detect that odorant.

Methods

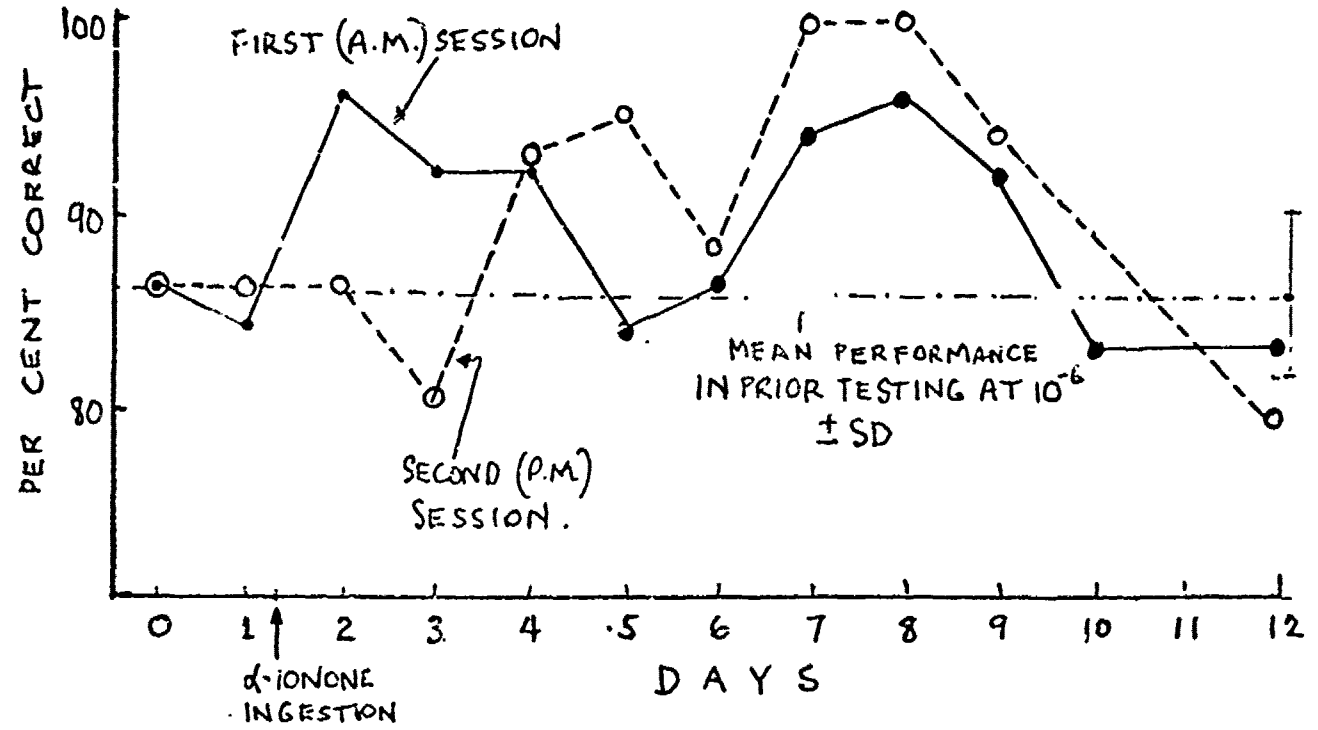
The dog used in this study was trained and tested according to the methods outlined in the previous report and summarized in the previous section of this report.

Performance was stabilized on a single concentration ($10^{-4.5}$) of alpha-ionone, 1 ml a-ionone was administered orally within a capsule, and sessions were continued at a rate of 2 a day (100 trials) until performance returned to baseline.

Results and Discussion

The performance of the dog over the test period is shown in Fig. 6. The first mean score obtained on the first day is the baseline score. The performance shows some oscillation but the striking and highly significant peak scores of 97% on day 7 (six days after ingestion of alpha-ionone), far exceed the performance score that the dog was previously able to obtain on this concentration of the test odorant. Performance returns to baseline on the 9th day following ingestion of alpha-ionone (and remained at that level for several days after).

The magnitude of this effect was unexpected, particularly in view of the arbitrary nature of the concentration of alpha-ionone both administered and presented as a test stimulus. The implications are several and include: (1) The performance of dogs in detecting of specific


100
90
80
PER CENT CORRECT
FIRST (A.M.) SESSION
SECOND (P.M.) SESSION.
MEAN PERFORMANCE IN PRIOR TESTING AT 10^{-6} ± SD
0 1 2 3 4 5 6 7 8 9 10 11 12
DAYS
α-IONONE INGESTION

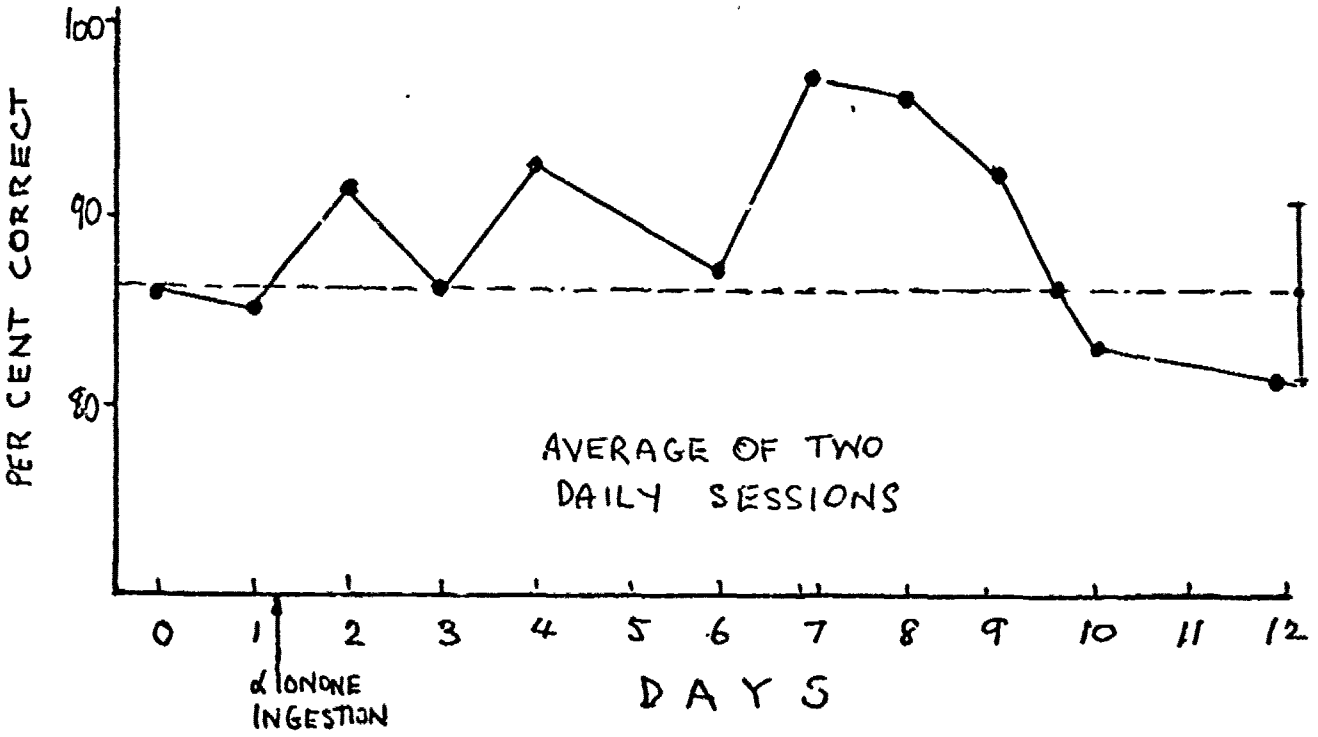

100
90
80
PER CENT CORRECT
AVERAGE OF TWO DAILY SESSIONS
0 1 2 3 4 5 6 7 8 9 10 11 12
DAYS
α IONONE INGESTION

Fig 6.

odors may be improved by administration of that odor. (2) If the effect is most specific for the odor administered it increases the probability that it is determined at the receptor level. This opens the possibility of using the effect as a tool to investigate the odor specificities of receptor sites. (3) There are possible implications of this effect in nutrition and in disease conditions. For example, in terminal cancer, anorexia is sometimes an added complication. It may arise as follows: In certain conditions diseased organs such as the liver may produce abnormal metabolic by-products which are both volatile and have unpleasant odors. These compounds enter the circulation and pass to the expiratory air flow by way of the lung. When they reach the nasal chamber following a meal the patient learns to associate the meal with unpleasant sensations. He may not necessarily identify the sensation as an odor. The effect, however, is to decrease food intake.

Although the mechanism of the effect and its degree of specificity have not been established in this study the time course of the effect suggests that a sensitization of receptor sites may occur on analogy with the immune response. This effect could result from the direction action of compounds in the blood stream on the receptors without requiring an intermediary gaseous step. Alternatively, the continuous presence of alpha-ionone molecules in the nasal cavity might provide a baseline concentration against which incoming molecules (which the dog sniffs) could summate. Normally, however, such an effect should lead to adaptation - not enhancement.

PART II

RELATION BETWEEN PROGESTERONE LEVELS AND PERFORMANCE OF FEMALE RATS ON AN ODOR DETECTION TASK

Introduction

In earlier annual reports and in a recent publication[1] we have shown that female rats - in contrast to males - show cyclic variations in performing an odor detection task. These fluctuations are associated with the phases of the estrous cycle are non-specific to the odor tested, maximal around ovulation and eliminated by ovariectomy and the induction of pseudopregnancy. We further found a linear correlation coefficient between circulating levels of estrogen and performance in detecting cyclopentanone, of .99 during the estrous cycle.

This surprisingly close correlation strongly suggests a casual relation and raised the possibility that progesterone levels might also correlate with performance but in the opposite direction. In other words, it is known that progesterone levels are high immediately following ovulation - the time during which performance levels began to decline.

To make this comparison the performance data derived from the earlier study measured on a scale of hours rather than days since hormonal levels alter rapidly during certain stages of the estrous cycle. The magnitudes of levels in circulating progesterone have been reported by Hashimoto et al. (1968)[2].

Method

Rats were tested in the two-choice odor discrimination apparatus described in the 1972 report on AFOSR Contract F44 620-70-C-0110. The odor was cyclopentanone at 10^{-3} of saturated vapor at 23°, the subjects were six three-month old female Long-Evans hooded rats (200-250 g) on a water deprivation schedule.

Rats were tests at 2.00, 3.00, 4.00, 9.00, 10.00 and 11.00 PM over a period equivalent to two complete estrous cycles. Each rat received a total of 480 trials. The data for each cycle were then combined and plotted against published data for the progesterone concentration in ovarian venous plasma during the estrous cycle.

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[1] Pietras, R. J. and Moulton, D. G. (1974) Hormonal influences on odor detection in rats: changes associated with the estrous cycle, pseudopregnancy, ovariectomy, and administration of testosterone propionate. Physiology and Behavior 12, 475-491.

[2] Endocrinol. 82, 333-341.

Results and Discussion

Figure 7 shows the results of the comparison. Both the actual and mean performance scores are plotted against levels of progesterone A. The general correspondence in the shapes of these curves is immediately apparent.

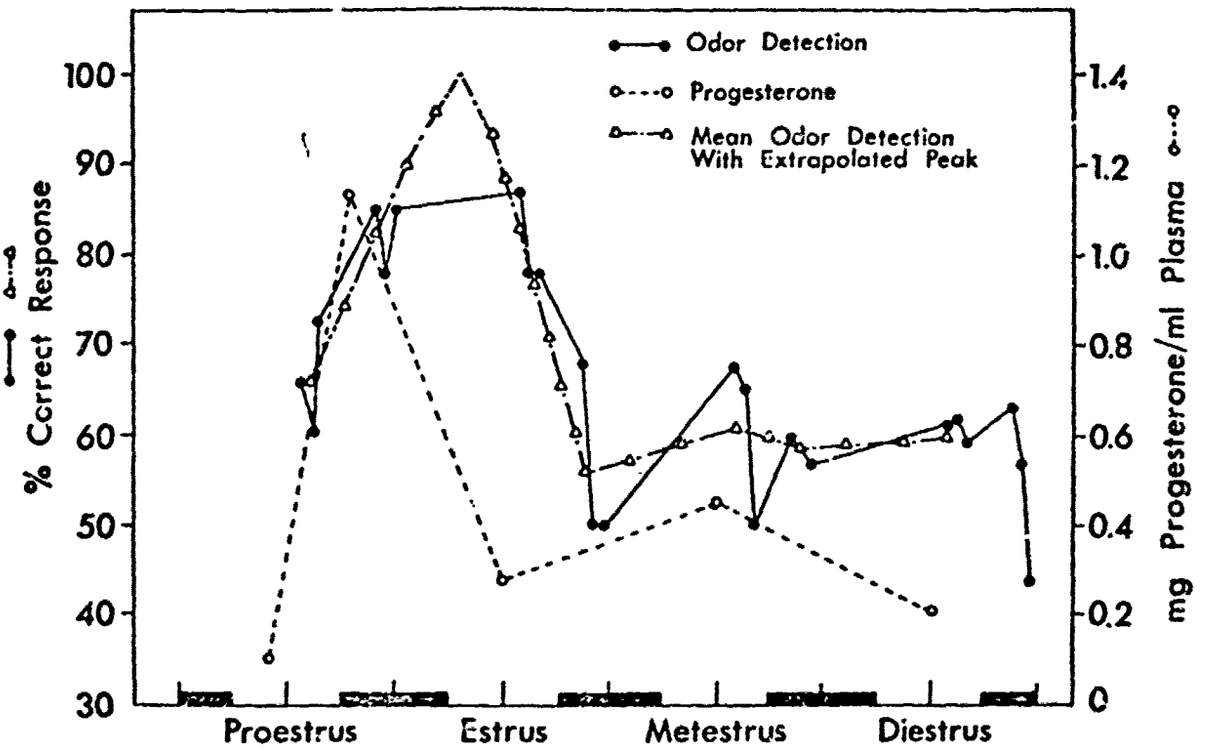

Figure 7. Comparison of mean and actual performance levels of six female rats detecting cyclopentanone (10^{-3}) during the estrous cycle with corresponding venous levels of Progesterone A.

As in the case of estrogen the peaks and troughs of the mean detection values and of progesterone A levels follows each other with a displacement in time. In this case the displacement for the main peak is in the order of 12 hours as compared to 19.8 hours in the case of estrogen. If, however, we assume the same delay applies it suggests that the peak in progesterone levels occurs some 7 hours after the peak in estrogen and in performance. In other words its rise corresponds with the beginning of the downward phase of performance.

To devise a more quantitative estimate of the degree of relatedness of these two variable and to determine whether progesterone A or some other derivative shows the closest relation linear regression

analysis was applied to paired points. The results are summarized below.

TABLE I

Compound	Correlation Coefficient	Significance level (p)
Progesterone A	.999	<.001
Progesterone B	.458	>.05
Progestin	.897	>.05

(Pregn-4-en-20α-ol-3-one)

Of these comparisons only the first is significant and the level of significance is high. Consequently it seems reasonable to suggest that there exists an antagonistic relation between estrogen and progesterone A. Rising estrogen levels correspond with rising detection performance. As progesterone levels rise and estrogen falls performance begins to fall.